In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference

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Although the use of pesticides in inner-city homes of the United States is of considerable magnitude, little is known about the potentially adverse health effects of such exposure. Recent animal data suggest that exposure to pesticides during pregnancy and early life may impair growth and neurodevelopment in the offspring. To investigate the relationship among prenatal pesticide exposure, paraoxonase (*PON1*) polymorphisms and enzyme activity, and infant growth and neurodevelopment, we are conducting a prospective, multiethnic cohort study of mothers and infants delivered at Mount Sinai Hospital in New York City. In this report we evaluate the effects of pesticide exposure on birth weight, length, head circumference, and gestational age among 404 births between May 1998 and May 2002. Pesticide exposure was assessed by a prenatal questionnaire administered to the mothers during the early third trimester as well as by analysis of maternal urinary pentachlorophenol levels and maternal metabolites of chlorpyrifos and pyrethroids. Neither the questionnaire data nor the pesticide metabolite levels were associated with any of the fetal growth indices or gestational age. However, when the level of maternal PON1 activity was taken into account, maternal levels of chlorpyrifos above the limit of detection coupled with low maternal PON1 activity were associated with a significant but small reduction in head circumference. In addition, maternal PON1 levels alone, but not *PON1* genetic polymorphisms, were associated with reduced head size. Because small head size has been found to be predictive of subsequent cognitive ability, these data suggest that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers who exhibit low PON1 activity. *Key words:* chlorpyrifos, exposure assessment, fetal growth, neonatal, paraoxonase gene and phenotype, pesticides, pregnancy, urinary biomarkers. *Environ Health Perspect* 112:388–391 (2004). doi:10.1289/ehp.6414 available via *http://dx.doi.org/* [Online 18 November 2003]

The potentially adverse health effects of indoor pesticide use on children have been raised as a concern in several recent publications (Davis and Ahmed 1998; Eskenazi et al. 1999; Slotkin 1999). Children are at higher risk of exposure due to their play close to the ground and their frequent hand-to-mouth behavior. Furthermore, infants exposed *in utero* and during early neonatal life are particularly vulnerable because of their rapid growth, cell differentiation, immaturity of metabolic pathways, and development of vital organ systems (Eskenazi et al. 1999; Landrigan et al. 1999).

Chlorpyrifos, an organophosphate, has been the most commonly used pesticide in the United States. Before the U.S. Environmental Protection Agency's (EPA) recent regulatory action to phase out residential uses of chlorpyrifos, domestic use was estimated to have accounted for nearly 50% of its applications (Nixon 2000). In New York State in 1997, the largest quantity of legally registered pesticides was applied in the boroughs of Manhattan and Brooklyn, and the most frequently used pesticide was chlorpyrifos (CP) (Thier et al. 1998). Whyatt et al. (2002), in a study of African-American and Dominican pregnant women in New York City, detected chlorpyrifos and diazinon in 100% of personal air monitor samples. In our study population, 3,5,6-trichloro-2-pyridinol (TCPy; a metabolite of chlorpyrifos) and 3-phenoxybenzoic acid (PBA; a pyrethroid metabolite) were each detected in more than 40% of prenatal urines (Berkowitz et al. 2003). Because the half-lives of these compounds are short (Leng et al. 1997; Nolan et al. 1984; Uhl et al. 1986), these data suggest that a large proportion of pregnant women in the United States may have been continuously exposed to low doses of these pesticides. It has also been shown that CP residues can persist for at least 2 weeks after application and that dermal contact with sorbent surfaces can further increase exposure (Gurunathan et al. 1998).

The toxicity of CP relies in part on acetylcholinesterase inhibition, which is the causal mechanism behind the acute neurotoxic syndrome observed at short-term high-dose exposure (Withney et al. 1995). Of greater concern are the findings from *in vitro* and animal studies that low-dose exposure to embryonic cells or animals *in utero* or in early postnatal life can produce neurochemical and neurobehavioral changes, including decreased DNA synthesis, decreased cell proliferation, changes in synoptic proliferation, and reflex impairment (Chanda and Pope 1996; Dam et al. 1999; Song et al. 1998; Withney et al. 1995). This has led to the suggestion that the developing brain provides a window of particular vulnerability to pesticide exposure during the fetal and childhood period (Dam et al. 2000; Eskenazi et al. 1999; Qiao et al. 2003; Slotkin 1999).

Paraoxonase (PON1) is an enzyme that acts as a phase-II detoxifying system for arylester type moieties, including organophosphate pesticide metabolites (Smolen et al. 1991). Specifically, PON1 can detoxify the chlorpyrifos oxon before it can inhibit acetylcholinesterase in the peripheral and central nervous systems (Furlong et al. 1988). PON1 also possesses more general antioxidant effects on lipids and other phenolic substances (Aviram et al. 2000).

The present study was set up to examine the effects of *in utero* pesticide exposure on fetal growth and neurodevelopment in a cohort of infants delivered at Mount Sinai Hospital in New York City. In this report we consider maternal exposure to pesticides, PON1 activity, and genetic susceptibility in relation to indices of growth and gestational age at birth among 404 infants.

Materials and Methods

The Children's Environmental Cohort Study is a prospective study that is following an ethnically diverse cohort of mother–infant pairs at Mount Sinai Hospital in New York City. Details regarding the study design and study procedures have been described previously (Berkowitz et al. 2003). Briefly, the mothers were recruited consecutively during early

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pregnancy from the Prenatal Clinic and two private practices at Mount Sinai Hospital from March 1998 to March 2002. The study includes only primiparas with singleton births. In addition, excluded were mothers who had their first prenatal visit after 26 weeks of gestation; mothers with serious chronic diseases such as diabetes, hypertension, or thyroid disease or those who developed a serious pregnancy complication that could affect fetal growth and development; and mothers who consumed more than two alcoholic beverages (wine, beer, hard liquor) per day or who used illegal drugs. Mothers and infants were also excluded if the child was born with a congenital malformation or severe prematurity (< 1,500 g or < 32 weeks of gestation). A total of 479 prenatal patients were recruited. Of these, 75 were excluded because of medical complications, very premature births, delivery of an infant with birth defects, inability to collect biologic specimens before birth, change of hospital or residence outside New York City, or refusal to continue to participate. The final sample size for this analysis comprised 404 births. This study was approved by the Institutional Review Board of the Mount Sinai School of Medicine.

A prenatal questionnaire was administered to the mothers during their third trimester to obtain information on pesticide and other environmental exposures, sociodemographic characteristics, maternal health, and lifestyle habits.

Participants' data were linked to a computerized perinatal database within the Department of Obstetrics, Gynecology, and Reproductive Science at Mount Sinai Hospital. From this database we obtained information on delivery characteristics and birth outcome, including birth weight, length, head circumference, gestational age, and infant sex. Standardized clinical techniques were used to measure birth weight, length, and head circumference.

Maternal blood samples were obtained during the third trimester at the time of routine venipuncture. Cord blood samples were obtained at birth. These samples were used to determine maternal and infant PON1 activity and *PON1* polymorphisms. Maternal urine samples were also obtained at the same time as the maternal blood samples. Three phenolic metabolites of pesticides were determined in urine: TCPy, PBA, and pentachlorophenol (PCP). TCPy, a CP metabolite, has been one of the most commonly detected pesticide metabolites. PBA is a possible metabolite of several pyrethroid insecticides, including sumithrin, permethrin, and cypermethrin. Sumithrin was used to spray against the West Nile Virus in New York City during the summer of 2000. PCP was widely used as a wood preservative until the 1970s and is also a metabolite of hexachlorobenzene and lindane [Agency for Toxic Substances and Disease Registry (ATSDR) 2001].

The analytic methods were adapted from reported methods (Chang et al. 1996; Hill et al. 1995; Nolan et al. 1984) and have been described previously (Berkowitz et al. 2003). Briefly, samples were hydrolyzed with acid followed by solid phase extraction. High-performance liquid chromatography (HPLC; Varian Associates, Sugarland, TX) on a C18 column was performed with ultraviolet detection (290 nm). In addition, diode array detection was used for confirmation at 2 additional wavelengths—273 and 302 nm for TCPy based on scans of external standards. For peaks with a concentration more than four times the limit of detection (LOD), we considered values missing if the confirmation spectra was not acceptable. The LOD was defined as three times the standard deviation of water blanks run with each batch over the course of the analysis (n = 44). The LODs were 11.0 μg/L for TCPy, 15 μg/L for PBA, and 20 μg /L for PCP. The proportion of our values below LOD was 57% for TCPy, 43% for PBA, and 76% for PCP. These LODs are slightly different from those reported previously (Berkowitz et al. 2003) because the sample size was larger for this study (n = 404) than for the previous report (n = 386). Urinary concentrations were corrected for creatinine levels and are expressed as micrograms per gram creatinine.

PON1 activity was assessed by measuring the hydrolysis of phenylacetate (added to plasma) to acetic acid and phenol, as previously described (Chen et al. 2002). The categorization of PON1 activity into low, medium, and high was based on its tertile distribution. The *PON1* genotypes were determined using restriction fragment length polymorphism (RFLP) or allele-specific polymerase chain reaction (PCR). *PON1* has two common polymorphisms in the coding region, *Q192R* and *L55M*, and three common polymorphisms in the promoter region at *–909, –162*, and *–108*. It has been shown that the predominant promoter polymorphism contributing to the phenotype (enzymatic activity) is *PON1-108* (Brophy et al. 2001; Chen et al. 2003). We have also shown that *L55M* may be an independent contributor to phenotype (Chen et al. 2003).

Covariates to be used in multivariate analyses were selected from variables known to be associated with either pesticide exposure or fetal growth. Only variables that were statistically significant (p < 0.05) were included in the final models. Generalized linear models were used to analyze the associations among pesticide exposure (assessed by questionnaire items as well as by urinary metabolite levels), PON1 activity, and birth weight; birth length; head circumference; and gestational age, adjusting for the selected covariates. The pesticide metabolite data were classified as below and above the LOD. PROC GLM in SAS (SAS Institute, Cary, NC) was used to fit the models.

Results

The study population consisted of 404 mothers and their infants. The patients were drawn predominantly from East Harlem, but also from other parts of New York City. The distributions of sociodemographic characteristics and reported pesticide use are given in Table 1. The women were relatively young, with 35.4% under age 20. The largest racial/ethnic group was Hispanics, who are predominantly Puerto Rican in our study population, followed by African-Americans and whites. The proportion of women who were single was 46.8%, with another 24.5% who were cohabiting with the infant's father. As a reflection of the young age distribution, 29.5% had not completed high school, although 49.9% were college graduates or had received some college education.

Almost half (46.2%) reported that they or a household member had used indoor pesticides during the pregnancy. When a composite index of indoor pesticide use was calculated on the basis of a positive response to either household or exterminator application, fumigation, or pesticide use in common areas, 71.5% were classified as having been potentially exposed (Table 1). No significant sociodemographic differences were seen for the latter estimate, although any reported pesticide exposure tended to be higher for younger women.

The medians and interquartile ranges for the urinary pesticide metabolites (uncorrected

Table 1. Distribution of maternal sociodemographic characteristics and pesticide use, Children's Environmental Health Study, Mount Sinai Hospital, 1998–2002 (total n = 404).

Characteristics	No. (%)
Maternal age (years)	
< 20	143 (35.4)
20–24	132 (32.7)
25–29	44 (10.9)
30–34	63 (15.6)
≥ 35	22 (5.4)
Race/ethnicity	
White	85 (21.0)
African American	112 (27.7)
Hispanic	201 (49.8)
Other[a]	6 (1.5)
Marital status	
Married	116 (28.7)
Living with the baby's father	99 (24.5)
Single/divorced/widowed/separated	189 (46.8)
Maternal education	
Lower/middle school	119 (29.5)
High school graduate	83 (20.6)
Some college	103 (25.6)
College graduate	98 (24.3)
Pesticide use by household member	186 (46.2)
Any reported indoor pesticide use	289 (71.5)

[a]Includes mixed race/ethnicity.

for creatinine) were 7.6 (1.6–32.5) μg/L for TCPy, 20.0 (2.4–69.8) μg/L for PBA, and 7.0 (2.0–19) μg/L for PCP. The respective medians (and interquartile ranges) for the creatinine-corrected metabolites were 11.5 (1.8–35.4) μg/g creatinine for TCPy, 19.8 (4.8–62.9) μg/g creatinine for PBA, and 8.0 (2.6–32.3) μg/g creatinine for PCP. Apart from higher TCPy levels for those who had completed at least a high school education as well as elevated PBA levels among those who were married or cohabiting and those who had completed high school or higher education level, no other significant associations were evident between sociodemographic characteristics and the pesticide metabolite levels.

Models including race/ethnicity, infant sex, and, where appropriate, gestational age were used to evaluate the associations between the questionnaire pesticide items and the pesticide metabolite levels with birth size and gestational age. Other potential covariates, such as active and passive cigarette smoking, prepregnancy body mass index, maternal weight gain, blood lead levels, and cesarean section delivery, were not included in the final models because they did not affect the results and only increased the variance. Marital status and educational levels were too closely correlated with race/ethnicity to be included in the analysis. Neither pesticide use by a household member nor any reported pesticide use was associated with adjusted birth weight, adjusted length, adjusted head circumference, or adjusted gestational age (Table 2). Similarly, no significant associations were observed between adjusted birth outcomes and TCPy, PBA, or PCP levels above and below the LOD (Table 3).

A significant positive trend was found between maternal paraoxonase activity and head circumference among the offspring of mothers whose TCPy levels were above the LOD (Table 4). A similar trend was seen for mothers with TCPy levels below the LOD, but this was not significant. The test for interaction among TCPy level, PON1 activity, and head circumference was not statistically significant ($p > 0.05$). Controlling for birth weight or birth length did not alter these results. Stratification by race/ethnicity showed similar trends across all three racial/ethnic groups, but the association was significant only for African Americans. Excluding preterm births did not affect the mean head circumferences either below or above the LOD.

A similar trend was observed with head circumference when maternal paraoxonase activity was considered alone (i.e., without level of TCPy); the adjusted means were 33.5 cm for the low PON1, 33.9 cm for medium PON1, and 34.1 cm for high PON1 activity ($p = 0.004$). No trends were seen for birth weight or birth length for TCPy or the other metabolite levels when maternal paraoxonase level was taken into account. Infant paraoxonase activity had no association with any of the fetal growth measures.

The race/ethnicity-adjusted correlation between *PON1-108* genotype and PON1 activity was 0.08 for the mothers and 0.24 for the infants. By incorporating the remaining linked variants, the correlation was improved somewhat for the mothers ($r = 0.12$) and slightly for the infants ($r = 0.28$).

The maternal genotypes were weakly associated with birth weight. For example, adjusted mean birth weight was 3,242 g for the maternal *PON1-108* homozygous low-activity allele, compared with 3,316 g for the combined homozygous and heterozygous high-activity allele ($p = 0.085$). No improvement was seen for the combined effects of the five maternal genotypes. No association was seen between infant genotype and birth weight. Furthermore, neither maternal nor infant genotypes were associated with head circumference or length at birth.

Table 2. Pesticide questionnaire data and adjusted mean ± SD of fetal growth and gestational age, Children's Environmental Health Study, Mount Sinai Hospital, 1998–2002.

	Pesticide use by household member		Any reported pesticide use	
Birth outcome	Yes ($n = 186$)	No ($n = 218$)	Yes ($n = 289$)	No ($n = 115$)
Adjusted birth weight[a] (g)	3,301 ± 457	3,277 ± 419	3,295 ± 461	3,269 ± 407
Adjusted birth length[a] (cm)	50.5 ± 2.5	50.6 ± 2.3	50.5 ± 2.6	50.7 ± 2.2
Adjusted head circumference[a] (cm)	33.8 ± 1.8	33.9 ± 1.6	33.8 ± 1.8	33.9 ± 1.6
Adjusted gestational age[b] (weeks)	39.4 ± 1.8	39.2 ± 1.7	39.4 ± 1.8	39.0 ± 1.6

[a]Adjusted for race/ethnicity, infant sex, and gestational age. [b]Adjusted for race/ethnicity and infant sex. None of the above differences was statistically significant.

Table 3. Adjusted mean ± SD of fetal growth and gestational age in relation to pesticide metabolite level, Children's Environmental Health Study, Mount Sinai Hospital, 1998–2002.

	TCPy		Phenoxybenzoic acid		Pentachlorophenol	
Birth outcome	< LOD ($n = 216$)	> LOD ($n = 171$)	< LOD ($n = 142$)	> LOD ($n = 185$)	< LOD ($n = 290$)	> LOD ($n = 92$)
Adjusted birth weight[a] (g)	3,284 ± 441	3,296 ± 434	3,295 ± 438	3,292 ± 432	3,289 ± 468	3,287 ± 408
Adjusted birth length[a] (cm)	50.4 ± 2.4	50.8 ± 2.4	50.7 ± 2.4	50.7 ± 2.3	50.6 ± 2.6	50.7 ± 2.2
Adjusted head circumference[a] (cm)	33.8 ± 1.7	33.8 ± 1.7	33.7 ± 1.7	33.9 ± 1.6	33.8 ± 1.8	34.0 ± 1.5
Adjusted gestational age[b]	39.3 ± 1.8	39.3 ± 1.7	39.4 ± 1.8	39.2 ± 1.8	39.2 ± 1.9	39.5 ± 1.6

[a]Adjusted for race/ethnicity, infant sex, and gestational age. [b]Adjusted for race/ethnicity and infant sex. None of the above differences was statistically significant.

Table 4. Adjusted mean ± SD of fetal growth indices by tertiles of maternal paraoxonase activity and TCPy level, Children's Environmental Health Study, Mount Sinai Hospital, 1998–2002.

	Birth weight[a] (g)		Birth length[a] (cm)		Head circumference[a] (cm)	
	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD	No.
TCPy < LOD						
Low PON	3,237 ± 456	76	50.3 ± 2.3	75	33.6 ± 1.8	76
Medium PON	3,255 ± 436	62	50.1 ± 2.2	62	33.7 ± 1.7	62
High PON	3,337 ± 444	71	50.3 ± 2.3	71	34.1 ± 1.7	70
TCPy > LOD						
Low PON	3,278 ± 395	47	50.9 ± 2.3	46	33.3 ± 1.5*	47
Medium PON	3,327 ± 406	57	51.0 ± 2.3	57	34.0 ± 1.5	57
High PON	3,270 ± 409	55	50.8 ± 2.4	55	34.1 ± 1.6	55

[a]Adjusted for race/ethnicity, infant sex, and gestational age. *$p = 0.014$.

Discussion

To our knowledge, there are no published reports on pesticide metabolites and birth outcomes. Although we found no direct effects of pesticide exposure based either on questionnaire responses or the pesticide metabolites on fetal growth or gestational age, we observed a significant albeit small decrease in head circumference when the levels of paraoxonase activity and TCPy were considered jointly. Although the test of interaction was not statistically significant, this is understandable because the trends were in the same direction both above and below the LOD for TCPy. This may be primarily a phenotypic effect, but the association was significant only among those whose TCPy level was above LOD.

An adverse effect on head circumference is biologically plausible because chlorpyrifos is a potential neurotoxin that can be detoxified by PON1 before it can inactivate acetylcholinesterase in the peripheral and central nervous system. It has been shown in animals that serum paraoxonase activity is inversely related to chlorpyrifos toxicity. Intravenous injection of paraoxonase before CP challenge has been shown to be protective in the rat (Costa et al. 1990), and *PON1* knockout

mice are more susceptible to organophosphate toxicity (Shih et al. 1998).

A previous study (Roy et al. 1994) reported a negative correlation between PON1 activity and birth weight. We found no significant relationship between PON1 activity or *PON1* genes and birth weight. Variations in the *PON2* gene have been associated with low birth weight in Trinidadian neonates of South Asian but not African ancestry (Busch et al. 1999). *PON2* genes were not analyzed in our study. However, we have shown that *PON1* and *PON2* are in linkage disequilibrium—that is, the two genes are not randomly associated on the same chromosome (Chen et al. 2003). Therefore, the aforementioned effect may not be caused by *PON2*.

The fact that the infant paraoxonase levels were not associated with birth outcomes is understandable. PON1 is not well expressed until after birth, and infant PON1 levels are much lower during the first year of life than maternal levels (Chen et al. 2003; Mueller et al. 1983). Specifically, the expression of PON1 in infants is approximately one-third that of their mothers (Chen et al. 2003). The lower PON1 activity in infants implies that infants may be more susceptible than adults to PON1-related toxicity, including exposure to organophosphate pesticides. In our population, both genotype and phenotype vary by race/ethnicity, suggesting that different populations may have different susceptibility to pesticides based on their national origin.

Head circumference has been shown to correlate with brain weight (Lemons et al. 1981). Both brain size and head circumferences are, in turn, predictive of IQ and cognitive ability (Lasky et al. 1981; Ounsted et al 1998; Rushton et al. 1996; Willerman et al. 1991). Brain volume has also recently been associated with attention-deficit/hyperactivity disorder (Castellanos et al. 2002). Furthermore, it has been well documented that even small increases in prenatal or early childhood exposures to such environmental toxicants as lead and polychlorinated biphenyls can be associated with significant decrements in IQ and academic achievement up to 11 years of age (Bellinger et al. 1992; Jacobson and Jacobson 1996). It is not known what effects the decrements in head circumference observed in this study may have on IQ or behavior. The infants, however, are being followed to assess any cognitive or behavioral deficits.

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